

# EVIDENCE FOR SHEAR STRESS-MEDIATED DILATION OF THE INTERNAL CAROTID ARTERY IN HUMANS

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## **Short Title:**

Flow-mediated dilation of carotid arteries

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**Abstract**

Increases in arterial carbon dioxide tension (hypercapnia) elicit potent vasodilation of cerebral arterioles. Recent studies have also reported vasodilation of the internal carotid arteries during hypercapnia, but the mechanism(s) mediating this extra-cranial vasoreactivity are unknown. Hypercapnia increases carotid shear stress, a known stimulus to vasodilation in other conduit arteries. To explore the hypothesis that shear stress contributes to hypercapnic internal carotid dilation in humans, temporal changes in internal and common carotid shear rate and diameter, along with changes in middle cerebral artery velocity, were simultaneously assessed in 18 subjects at rest and during hypercapnia (6% CO<sub>2</sub>). Middle cerebral artery velocity increased significantly (69±10 to 103±17cm/s, P<0.01) along with shear in both the internal (316±52 to 518±105 1/s, P<0.01) and common (188±40 to 275±61 1/s, P<0.01) carotids. Diameter also increased (P<0.01) in both carotid arteries (Internal: +6.3±2.9%; Common: +5.8±3.0%). Following hypercapnia onset, there was a significant delay between the onset of internal carotid shear (22±12secs) and diameter change (85±51secs). This time-course is associated with shear-mediated dilation of larger conduit arteries in humans. There was a strong association between change in shear and diameter of the internal carotid (r=0.68, P<0.01). These data indicate, for the first time in humans, that shear stress is an important stimulus for hypercapnic vasodilation. The combination of a hypercapnic stimulus and continuous non-invasive high-resolution assessment of internal carotid shear and dilation may provide novel insights into the function and health of the clinically important extra-cranial arteries in humans.

**Keywords:**

Cerebral blood flow, carotid artery, carbon dioxide, shear stress, vasodilation, flow-mediated dilation.

## Introduction

Small cerebral arteries and arterioles (e.g. pial vessels) respond rapidly to changes in their metabolic milieu and are highly sensitive to the partial pressure of arterial carbon dioxide (PaCO<sub>2</sub>).<sup>1</sup> Vasomotor responsiveness to PaCO<sub>2</sub>, termed CO<sub>2</sub> reactivity, is integral to stabilizing blood pH levels, while previous studies have associated lower CO<sub>2</sub> reactivity to increased cardiovascular and all-cause mortality.<sup>2</sup> Recent MRI studies in humans have revealed that vasomotor changes also occur in the middle cerebral (MCA)<sup>3-5</sup> and basilar arteries<sup>6</sup> across the hypo- and hypercapnic range. These studies demonstrate the involvement of larger cerebral arteries in the PaCO<sub>2</sub> reactivity response (reviewed by Hoiland and Ainslie<sup>7</sup>), findings consistent with well-controlled animal studies.<sup>8</sup> Other recent studies employing Duplex ultrasound have investigated *extra*-cranial artery responses during hypo- and hypercapnia.<sup>9, 10</sup> These studies provide direct evidence that changes in end-tidal CO<sub>2</sub> (PETCO<sub>2</sub>) are associated with directionally similar, and dose dependent, changes in internal carotid artery (ICA) diameter. The mechanism(s), however, mediating these changes in *extra*-cranial ICA diameter remain unclear.

Significant and rapid changes in *extra*-cranial artery blood flow occur across the hypo- and hypercapnic range.<sup>9-11</sup> In peripheral conduits such as the radial and brachial arteries, such changes in flow- and attendant arterial shear stress represent potent vasoactive stimuli.<sup>12, 13</sup> While Pohl *et al.*<sup>14</sup> and others<sup>15</sup> were the first to identify that flow-mediated dilation (FMD) is endothelium-dependent, it is now well established that this phenomenon occurs in humans and that nitric oxide (NO) plays a significant role.<sup>16-19</sup> The widely used FMD test<sup>13</sup> relies on dilation of small arteries and arterioles in the limbs, as a consequence of cuff-induced ischemia, to induce an increase in upstream conduit artery shear stress and dilation. In the context of these studies, it is conceivable that rapid and profound dilation of intracranial vessels in response to hypercapnia induces *extra*-cranial (ICA) dilation as a consequence of increased shear stress.

The aim of this study was to identify whether hypercapnia induces shear-mediated dilation in the carotid arteries. Using high-resolution Duplex ultrasound combined with novel edge-detection software, we assessed simultaneous common carotid artery (CCA) and ICA dilator responses evoked by hypercapnia. We tested the hypotheses that: i) hypercapnia would induce significant increases in CCA and ICA shear rate, ii) vasodilation of the vessels would occur secondary to this increase in shear rate, and iii) there would be a strong association between the magnitude of shear and subsequent diameter change in carotid arteries.

## **Methods**

### Subject Characteristics

Eighteen participants were recruited (9 females, 9 males; age  $26\pm 4$  yrs, height  $1.75\pm 0.12$ m, weight  $69\pm 12$ kg and BMI  $22\pm 2$  kg/m<sup>2</sup>). Participants who had a history of cardiovascular, musculoskeletal or metabolic disease; were smokers (or <6 months cessation); or taking medication of any kind, were excluded. To control for the effect of changes in circulating hormones on vascular function, all females were assessed in the follicular phase of the menstrual cycle.<sup>20</sup> The study was approved by the University of Western Australia's Human Research Ethics Committee and conformed to the standards outlined in the Declaration of Helsinki. Participants were informed of all experimental procedures and any potential associated risks. Written informed consent was obtained from all participants prior to commencement of the study.

### Study Design

Subjects attended the laboratory having fasted for a minimum of 8 hours and abstained from alcohol, caffeine and vigorous exercise for at least 24 hours. Once instrumented, participants lay

supine for 10 min. All data were continuously recorded, beginning with a 2 min baseline period prior to inhalation of 6% CO<sub>2</sub>, 21% O<sub>2</sub>, balance nitrogen, for 4 minutes, followed by a 1 min recovery period. During this time, simultaneous assessment of intra-cranial velocity (measured by transcranial Doppler of the left MCA) and beat-by-beat extra-cranial blood flow (measurement by Duplex ultrasonography of the left ICA and CCA) was assessed along with beat-to-beat arterial pressure (Finapres Medical Systems, Amsterdam, Netherlands). To minimize the influence of turbulent flow on vascular responsiveness, the CCA and ICA were imaged at least 2cm below and above the point of bifurcation respectively. All studies were performed in a quiet, temperature-controlled laboratory.

## Experimental Measures

### *Transcranial Doppler and systemic hemodynamics*

Middle cerebral artery velocity was measured using a 2 MHz pulsed ST3 transcranial ultrasound system (Spencer Technologies, Seattle, USA). The MCA was identified using search techniques described in detail elsewhere.<sup>21</sup> A Marc 600 headframe (Spencer Technologies, Seattle, USA) was secured around the participant's head to allow for adjustments to the insonation angle until an optimal M-mode image was found. Raw analogue MCA cerebral velocity was exported from PowerLab to LabChart 7 for *post-hoc* analysis. The PETCO<sub>2</sub> was measured in all subjects via a sampling tube connected to a Hans Rudolph mask via an online and calibrated gas analyzer (ML206; ADInstruments, Sydney, Australia). Beat-to-beat reconstructed brachial blood pressure, mean arterial pressure and heart rate was measured using a Finometer Pro (Finapres Medical Systems, Amsterdam, Netherlands).

### *Vascular Ultrasonography*

The CCA and ICA were assessed on the left side by two expert vascular sonographers. The

diameter and blood flow velocity of each artery were simultaneously recorded throughout the hypercapnic protocol using two identical 10-MHz multi-frequency linear array probes attached to a high-resolution ultrasound machine (T3200; Terason, Burlington, MA). The CCA and ICA were identified and the images were optimized in accordance with recent methodological guidelines.<sup>22</sup> Simultaneous recordings began after optimization of the longitudinal B-mode image of the lumen-arterial walls. Concurrently, Doppler velocity assessments were collected using the lowest possible insonation angle (always  $<60^\circ$ ). Our group have previously reported high reproducibility for the assessment of ICA diameter, with a within day coefficient of variation of 1.5%.<sup>10</sup>

#### Data Analysis

The reactivity of MCAv, ICA blood flow and CCA blood flow with elevations in PETCO<sub>2</sub> was analyzed by averaging 30 secs of baseline data to that of the peak velocity/blood flow responses and PETCO<sub>2</sub> obtained during the last 30 secs of hypercapnia. Linear regression analysis was then performed to calculate reactivity slopes.

All data from LabChart (MCAv, raw brachial and mean arterial pressure, raw PETCO<sub>2</sub> and O<sub>2</sub> and PETCO<sub>2max</sub> and O<sub>2max</sub>) were exported as 1-sec averaged bins into Excel. Analysis of CCA and ICA diameter and flow were performed using custom-designed edge-detection and wall-tracking software (BloodFlow Analysis, Version 5.1) – an approach that is independent of investigator bias and has previously been comprehensively described and validated.<sup>23, 24</sup> From synchronized diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity) and shear rate ( $4 \times$  mean blood velocity/vessel diameter) were calculated at 30Hz. Vascular data were then interpolated from 30Hz to 1Hz and exported into an excel spreadsheet. The LabChart and vascular data were time-aligned in Excel for subsequent analysis using in-house carotid shear-

mediated dilation software (General Purpose Data Graphing Software, Version 1).

*Carotid shear-mediated dilation software*

All data were passed through a two-stage filtering process; a median filter (with a rank of 7) was applied to the parameter data, followed by passage through a Savitzky-Golay finite impulse response Smoothing Filter with a window size of 13 data points and a polynomial order of 1. These filters removed high frequency noise to reveal the underlying lower frequency physiological response profiles. All subsequent analysis was performed using this graphed, filtered data. The following variables were automatically detected and calculated by the software: 1. Baseline: the median value of the 2 min baseline period preceding hypercapnia; 2. Peak response: the auto-detected maximum value of the filtered data identified following the onset of CO<sub>2</sub> and; 3. Relative (%) change: change from Baseline to Peak, calculated as  $((\text{Peak} - \text{Baseline}) / \text{Baseline}) \times 100$ . In addition, a thresholding algorithm was applied to each data array (e.g., ICA shear, ICA diameter, CCA shear, CCA diameter), which identified threshold points. These thresholds were defined as the point at which each variable began to systematically increase, above the baseline level, following the application of the CO<sub>2</sub> stimulus. The threshold point was calculated as:

$$= [\text{Max value} - \text{Min value}] \times (\text{Variation Factor } \%) + \text{Baseline Median Value}$$

[Max Value - Min Value] was across the whole study and took into account the maximum variation in each study. Two researchers analyzed the variables using a standard Variation Factor of 10% in the equation across all studies. This Variation Factor was chosen to ensure that the variable had increased to a point that represented a definitive deviation from baseline, which also exceeded fluctuations associated with cardiac and respiratory cycles. Once the software had automatically detected the threshold points, they were depicted on the raw data array and visually inspected to ensure they met the following criteria: a) the algorithm-detected threshold point occurred prior to the peak value and, b) the variable did not decrease below the algorithm-detected threshold point

prior to the peak value occurring. Of the 90 responses analysed, 78 met the agreed criteria and their automatically detected points were accepted and not modified. In the remaining 12 cases (13%), the threshold points were manually adjusted independently by the two analysers to a point where each deemed there was a clear deviation from baseline values that met the above criteria. The two analysers independently came to the conclusion that the same 12 responses needed manual adjustment, highlighting the robustness of our criteria in determining adequate threshold detection. The mean of these manually assessed points was entered in the analysis. The coefficient of variation for the analysis of the threshold points detected using the above systematic approach was 1.6%. The CV for the 12 manually adjusted files between independent observers was 11.3%.

#### *Statistical Analysis*

Statistical analysis was performed using SPSS 21.0 (SPSS, Chicago, IL) software. All CCA and ICA shear and diameter data were compared within- and between subjects (for analysis of gender related differences) using repeated measures 2 way ANOVA, with Bonferroni corrected post hoc *t*-tests. MCAv and hemodynamic data were compared via 2-tailed paired (base v peak data) and unpaired (gender) *t*-tests. Threshold point data (time dimension in seconds) was compared using a 1 way ANOVA with Bonferroni corrected post hoc *t*-tests. A multivariate ANOVA with Bonferroni corrections was used to compare MCAv, CCA and ICA CO<sub>2</sub> reactivity values. Bivariate correlations were performed between % change from baseline to max values for ICA and CCA diameter and shear. Finally, a correlation was performed between absolute change in mean arterial pressure and ICA and CCA diameters in 30 sec increments from baseline. Statistical significance was assumed at  $P < 0.05$ . All data are reported as means  $\pm$ SD.

## Results

In response to 6% CO<sub>2</sub> inhalation, PETCO<sub>2</sub> increased from 41±5 at baseline to a peak of 54±3mmHg (P<0.01) while PETO<sub>2</sub> also increased from 120±5 to 135±2mmHg (P<0.01). Heart rate (58±9 to 73±11bpm, P<0.01) and mean arterial pressure (92±9 to 104±11mmHg, P<0.01) were also elevated with hypercapnia. Average MCA cerebrovascular reactivity was 2.44±0.58 cm.s/mmHgPETCO<sub>2</sub>, while ICA and CCA reactivity were 14.0±7.4 and 16.1±5.8 ml.min/mmHg PETCO<sub>2</sub> respectively.

### *Arterial blood flow, shear rate and diameter responses*

There was an increase in MCAv by 49±19% from baseline (69±10 to 103±17 cm/s, P<0.01). Accordingly, blood flow through both the CCA and ICA increased significantly during hypercapnia (70±43% and 90±40% respectively, both P<0.01); therefore, the relative increase in ICA flow was greater than that in the external carotid artery. Shear rate was higher at baseline in the ICA compared to the CCA (P<0.01, Table 1). Similar to the blood flow responses, shear increased through both arteries during hypercapnia (both P<0.01, Figure 1), with a greater relative increase observed in the ICA when compared to the CCA (P<0.01, Table 1). Finally, with no differences between the arteries (P=0.53, Figure 2), the CCA dilated by 5.8±3.0% and the ICA by 6.3±2.9% (both P<0.01).

### *Time course of arterial hemodynamic responses*

MCAv increased 18±9 secs after the onset of hypercapnia (Figure 3), followed by ICA (22±12 secs) and CCA shear (32±44 secs), however there were no significant differences between the above variables. Dilation of the CCA occurred at 53±30 secs, significantly earlier than the ICA (85±51 secs, P<0.05). Finally, a difference was evident between the onset of change in shear and the onset

of change in diameter of the ICA ( $P<0.01$ ), with an average latency period of  $64\pm 53$  secs. No difference was evident between the onset of shear and diameter in the CCA ( $P=0.12$ ).

#### *Influence of gender on responses*

While no difference was evident between baseline MCAv values ( $P=0.07$ ), peak MCAv responses were higher in females compared to males ( $114\pm 17$  vs  $92\pm 7$  cm/s,  $P=0.002$ ). A 2-way ANOVA between CCA and ICA baseline and peak shear rate data revealed a significant difference for gender ( $P<0.001$ ). Post hoc tests revealed that while there was no difference in CCA shear rate at baseline between the groups, peak CCA shear rates were higher in the female group ( $P=0.008$ ). Baseline ICA shear was higher in females, along with peak shear responses ( $P=0.009$  and  $P<0.001$  respectively). No difference was evident between CCA and ICA baseline or peak diameters between the groups (Table 1). No difference was evident between groups for the threshold point data. Finally, females displayed greater MCA cerebrovascular reactivity compared to males ( $2.84\pm 0.50$  vs  $2.04\pm 0.32$  cm.s/mmHg PETCO<sub>2</sub>,  $P=0.001$ ), however there was no difference between genders in ICA or CCA reactivity.

#### *Relationships between selective variables of interest*

The relative increase in shear between the CCA and ICA were significantly correlated ( $r=0.68$ ,  $P<0.01$ ). A significant correlation was also evident between the % change in pooled CCA and ICA diameter and shear rate ( $r=0.43$ ,  $P<0.01$ , Figure 4A). Interestingly, further analysis revealed a significant association between ICA shear and diameter ( $r=0.68$ ,  $P<0.01$ , Figure 4C). The correlation between % change in CCA diameter and shear rate in the CCA was not significant ( $r=0.15$ ,  $P<0.55$ , Figure 4B). MAP increased  $51\pm 73$  secs after the onset of hypercapnia, and there was no association between threshold time points for increase in MAP and ICA diameter or MAP and CCA diameter ( $r=-0.21$  and  $r=0.17$ , respectively). However a positive correlation was evident

between change in MAP (mmHg) and ICA ( $r=0.56$ ,  $P<0.01$ ) and CCA diameter (mm) ( $r=0.53$ ,  $P<0.01$ ). Finally, no association was evident between time to peak for MAP and CCA dilation ( $r=0.31$ ,  $P=0.22$ ), or dilation of the ICA ( $r=0.26$ ,  $P=0.30$ ).

## Discussion

The primary aim of this study was to examine the shear rate and diameter responses of the extra-cranial cerebral arteries, the ICA and CCA, during transient hypercapnia. Our principle novel findings are: i) both the ICA and CCA dilate significantly in response to hypercapnia, ii) changes in artery velocity and shear in response to hypercapnia occurred first in the MCA, followed by the ICA and then the CCA iii) there was a significant time delay between the hypercapnia-induced onset of increase in shear and the onset of increase in diameter in both the ICA and CCA, characteristic of shear-mediated dilation (but not direct CO<sub>2</sub> mediated diameter change), and iv) there was a strong association between changes in shear and diameter in the ICA, but not the CCA. These findings are suggestive that increases in shear play an important role in ICA dilation during hypercapnia and also suggest distinct regulatory differences between the ICA and CCA.

Although not a universal finding,<sup>5, 11</sup> our ICA findings are broadly consistent with recent studies,<sup>9, 10</sup> adding robustness to the notion of ICA dilation during hypercapnia. Differences in measurement approaches (manual vs automated) may explain these between-study differences (reviewed in:<sup>10</sup>). Our findings are the first to show that the CCA also dilates to a similar extent to the ICA during hypercapnia. By characterizing the time-course of change in MCA velocity and ICA and CCA shear and diameter following application of hypercapnia, this study provides insight into the mechanisms responsible for extra-cranial hypercapnic dilation in humans. Although conceivable that extra-cranial arteries are directly affected by changes in arterial CO<sub>2</sub> tension, or reflex impacts of

hypercapnia (i.e. MAP), it is logical that such mechanisms would be rapidly evoked following application of the CO<sub>2</sub>/pH stimulus, with minimal delay between the hypercapnia and the onset of dilation. For example, pial vessels are intrinsically sensitive to CO<sub>2</sub> via changes in extra-cellular pH, and respond by dilating instantaneously.<sup>1, 25, 26</sup> We speculate that this is reflected in the present study by the rapid increase in MCA velocity.<sup>27</sup> However, the ICA (and CCA) did not dilate with the same temporal dynamics as previously reported in the pial vessels. This dissimilar reactive behavior to hypercapnia suggests distinct regulation and provides evidence that ICA and CCA vasodilation is not solely mediated via intrinsic CO<sub>2</sub> sensitivity.

We hypothesized that the dilator responses of the ICA might be shear-mediated, as is the case in peripheral conduit arteries when distal arteriolar beds dilate.<sup>13</sup> The latencies we observed between the onset of increases in ICA shear and diameter in the present study are congruent with *in vivo* cerebral flow-mediated dilation in animals<sup>28</sup> and are also consistent with shear-mediated dilation in peripheral arteries in humans.<sup>23</sup> We also observed a correlation between % change in shear and diameter in the ICA. Cumulatively, these results are suggestive of a shear-mediated role in ICA dilation. As there was a correlation between change in ICA dilation and MAP, the role of blood pressure in ICA dilation cannot be dismissed. However, no correlation was evident between either the threshold time points or the time to peak for ICA dilation and MAP, as would be expected if there was a passive effect of pressure on artery dilation. Increases in intra-arterial pressure have typically been associated with the activation of vasoconstrictive mechanisms such as the “myogenic reflex” in peripheral arteries.<sup>29, 30</sup> To our knowledge there is no data investigating this in relation to the ICA in humans, however, if it occurred in the present study it would result in an underestimation of our % dilation results. In addition, increases in blood pressure can directly increase blood velocity. This may have affected vasomotor tone via increased shear, consistent with our primary findings. Finally, previous reports of ICA vasoconstriction during hypocapnia occurred

in the presence of slight increases in BP,<sup>9, 10</sup> which argues against the role of BP as a regulator of ICA vasodilation in the present study.

A novel aspect of the present study was the simultaneous assessment of both ICA and CCA diameter and shear changes within subjects in response to hypercapnia. Interestingly, the magnitude of dilation in the ICA and CCA were not highly correlated, despite close correlations between the eliciting shear rate stimuli within-subjects ( $r=0.68$ ). If flow and shear were principal determinants for vasomotor responses in both the CCA and ICA, similar diameter responses should have manifested. Yet, ICA dilation was strongly correlated with shear, whereas the CCA was not. Furthermore, both arteries were imaged simultaneously on the same side, meaning the prevailing blood pressure and other systemic stimuli were identical, yet distinct within-subject vasomotor responses were observed between the arteries. These findings suggest regulatory differences in the vasomotor control between the CCA and ICA, with the latter demonstrating greater shear dependence. Possible reasons for this difference in regulation of the CCA and ICA may relate to structural differences between the arteries. In the current study, the CCA was significantly larger, with a lower shear rate compared to the ICA. It is known that artery size influences the shear stimuli and functional responsiveness of an artery, which was potentially evident in the current study.<sup>31</sup> In addition, a previous MRI study has reported greater distensibility in the CCA compared to the ICA within-subjects.<sup>32</sup> This raises the interesting notion of the CCA's potential role in dampening the pressure waveform prior to the ICA and cranial vessels. In addition, if the ICA is stiffer than the CCA (i.e. displays lower distensibility), it would be more susceptible to the mechanical stressors of flow and pressure, and therefore may exhibit greater sensitivity to changes in shear rate than the CCA. If indeed the CCA is not as shear sensitive, possible alternative vasodilation mechanism/s include blood pressure mediated vessel distention, and changes in cardiac output and/or sympathetic activity.

## Gender differences

One unexpected finding of the present study was the greater MCAv CO<sub>2</sub> reactivity response in females compared to males. This gender difference in CO<sub>2</sub> reactivity is consistent with some previous studies,<sup>11, 33</sup> but not all.<sup>34</sup> In the present study, no difference was evident in ICA or CCA reactivity between males and females. This interesting observation suggests that the sex-related differences in MCA reactivity are a result of either i) methodological issues relating to the determination of MCA reactivity from velocities, which does not account for potential sex differences in the magnitude of change in MCA diameter, or ii) different regulation between the intra-cranial and extra-cranial vessels. The physiological significance of these sex differences remains unknown but may relate to artery size, which has impacts on functional responsiveness.<sup>31</sup>

Our findings have potential clinical relevance. Flow-mediated dilation of the brachial and femoral arteries, introduced by Deanfield and Celermajer<sup>13</sup> has been technically enhanced<sup>24</sup> and formalized as a measure of *in vivo* endothelial function and pre-clinical atherosclerosis in the past 2 decades.<sup>35</sup> The assessment of FMD is largely mediated by NO,<sup>19</sup> strongly correlates with coronary artery function,<sup>36</sup> and independently predicts cardiac events (reviewed by Green *et al*<sup>37</sup>). A 1% decrease in FMD is associated with an 8-13% increase in cardiovascular event risk.<sup>38, 39</sup> Thus, FMD has become a useful research and clinical tool providing insight into conduit artery function and health in humans. No such *in vivo* test has been specifically developed to provide insight into *cerebrovascular* function, physiology and risk, but it is conceivable that ICA shear mediated dilation may provide a window on the health of the cerebrovascular endothelium. Indeed, there is a positive association between greater arterial stiffness and microvascular brain disease and cognitive impairment<sup>40-42</sup> and a lower ICA dilation in response to a standardized shear-mediated stimulus may

therefore provide a useful marker of cerebrovascular function and future risk. Future studies will be necessary to test this proposal.

Although our findings are suggestive of hypercapnia-induced shear mediated ICA dilation, we did not infuse intra-arterial antagonists of endothelium-derived substances such as nitric oxide. Future studies, particularly in animal models, involving more invasive approaches, or the modulation of shear via carotid ligation, would strengthen the notion of shear-mediated extra-cranial artery dilation. Previously, it has been demonstrated that CO<sub>2</sub> reactivity is unaffected by prostaglandins, a known endothelium derived relaxing factor, through utilization of multiple cyclooxygenase inhibitors.<sup>10, 43</sup> While the precise role of NO remains controversial,<sup>44-46</sup> the lack of effect of other endothelium derived relaxing factors<sup>10, 43</sup> provides support that NO may be an important factor driving endothelium-mediated responses in the cerebral circulation of humans. It is also possible that CO<sub>2</sub> directly influenced the extra-cranial arteries in the current study, however we consider this unlikely since the timecourse of the vascular responses was delayed and consistent with shear-mediated adaptation, as discussed above. Future studies may also benefit from different approaches to the application of hypercapnia, for example employing a higher level of CO<sub>2</sub> over a shorter period of time to elicit a rapid increase in arterial shear and subsequently diameter, independent of the confounding influence of longer exposure to CO<sub>2</sub>. In the present study we did not independently manipulate or clamp changes in CO<sub>2</sub> or O<sub>2</sub>, and this may contribute to some of the variation between-subjects. Indeed, we observed an increase in O<sub>2</sub> during the hypercapnic period (120±5 to 135±2 PETO<sub>2</sub> mmHg, P<0.01). However, this represents a small change, and, as the cerebrovasculature is relatively insensitive to such small changes in the hyperoxic range,<sup>9</sup> changes in cerebral blood flow or the function of the measured arteries seem unlikely. Nonetheless, future studies should consider strictly controlling PETCO<sub>2</sub> and PETO<sub>2</sub> for example via end-tidal forcing systems similar to those used in previous studies.<sup>9, 10</sup> An additional limitation, inherent in the use of

transcranial Doppler, is the assumption that the diameter of the imaged vessel does not change.<sup>7</sup> We were unable to examine and compare the time course of velocity and diameter change between the intra- and extra-cranial arteries and there are no current technologies that allow for the temporal resolution to do so accurately.

### **Perspectives**

In summary, we report significant vasodilation in both the CCA and ICA in response to hypercapnia. By systematically determining the time latencies of cerebral blood velocity and diameter responses, we provide insight into the underlying mechanisms of extra-cranial vasodilation. Our findings suggest that shear stress is an important stimulus for hypercapnic ICA vasodilation in humans, secondary to the dilation of distal cerebral arterioles. The combination of an easily administered and standardized hypercapnic stimulus, with continuous high resolution assessment of internal carotid shear and shear-mediated dilation, may provide important insights into the function and health of the clinically important extra-cranial arteries in humans.

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### **Conflict of Interest/Disclosure**

None

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## **Novelty and Significance**

### **1) What is New**

- This is the first study to assess the temporal dynamics of changes in velocity and diameter in the internal and common carotid arteries simultaneously, and on the same side, during hypercapnia in humans.
- There was a significant time delay between the hypercapnia-induced onset of increase in shear rate and the onset of increase in diameter in both the internal and common carotid arteries, characteristic of shear-mediated dilation, but not direct CO<sub>2</sub> mediated diameter change.
- There was a strong association between changes in shear and diameter in the internal carotid artery.

### **2) What is Relevant?**

- Flow-mediated dilation of peripheral conduit arteries is a useful research and clinical tool that provides insight into artery function and cardiovascular health in humans.
- No *in vivo* test has been developed to provide insight into pre-clinical cerebrovascular function, physiology and risk.
- The findings of this study suggest that a standardized test of internal carotid shear-mediated dilation may provide a window on the health of the cerebrovascular endothelium.

## **Summary**

Our findings strongly support the notion that internal carotid artery dilation may be dependent upon increases in blood flow and shear and that shear stress is an important stimulus for hypercapnic vasodilation of the internal carotid in humans, secondary to the dilation of distal cerebral arterioles. These findings warrant the further investigation and development of a standardized hypercapnic stimulus, with continuous high resolution assessment of internal carotid shear and shear-mediated dilation, that may provide important insights into the function and health of the clinically important extra-cranial arteries in humans.

## Figure Legends

### Figure 1

A representative response showing internal (ICA) and common carotid artery (CCA) shear rate and diameter, middle cerebral artery velocity (MCAv) and partial pressure of end-tidal carbon dioxide (PETCO<sub>2</sub>) change during 2 mins of baseline immediately followed by 4 mins of hypercapnia (6%CO<sub>2</sub>). Threshold points are marked by vertical grey dashed lines.

### Figure 2

Absolute common (CCA) (A) and internal carotid artery (ICA) (B) diameter change from baseline to peak in response to hypercapnia (6% CO<sub>2</sub>). A 2 way ANOVA revealed a significant difference between pre and post diameters within arteries. There was no difference in the %change between arteries. \* significantly different from baseline P<0.01. Data are ± SD.

### Figure 3

The time-course of arterial hemodynamic responses from the onset of hypercapnia (6% CO<sub>2</sub>). A 1 way ANOVA revealed a significant difference between the onset of ICA shear and diameter, with an average latency period of 64±53 secs. There was also a difference between the onset of dilation between the ICA and CCA. Data are ± SD.

### Figure 4

Pearson's correlations between % change in pooled common (CCA) and internal carotid artery (ICA) diameter and shear rate (A), CCA diameter and shear (B) and ICA diameter and shear (C). There was a significant correlation between %change in shear and diameter in the pooled CCA and ICA data (P<0.01), and the ICA (P<0.01). No association was evident in the CCA.

Table 1. Baseline, peak response and % change from baseline for arterial shear rate and diameter data

	Baseline			Peak Response			% Change		
	Average (n=18)	Female (n=9)	Male (n=9)	Average (n=18)	Female (n=9)	Male (n=9)	Average (n=18)	Female (n=9)	Male (n=9)
CCA Shear Rate (1/s)	188±39.7	203±34	173±41	275±61*	311±51*	239±50*†	47±23	54±25	40±20
ICA Shear Rate (1/s)	316±52‡	347±38	286±49†	518±105*‡	597±55*	438±78*†	65±26	75±29	54±19
CCA Diameter (mm)	6.39±0.56	6.08±0.45	6.69±0.51	6.75±0.53*	6.45±0.38*	7.05±0.50*†	5.8±3.0	6.2±3.2	5.4±2.9
ICA Diameter (mm)	4.64±0.55‡	4.36±0.60	4.92±0.34	4.93±0.57*‡	4.67±0.65*	5.19±0.33*†	6.3±2.9	7.0±3.6	5.6±1.8

Values are means ± SD. CCA, common carotid artery; ICA, internal carotid artery. \*Significantly different from baseline; †Significantly different between Female and Males; ‡Significantly different between CCA and ICA.

